

**MODULATION OF GUT MICROBIOTA COMPOSITION AND
SHORT CHAIN FATTY ACID PRODUCTION BY JABOTICABA
EXTRACT IN DMH-INDUCED RATS**

***PENGARUH EKSTRAK JABOTICABA TERHADAP KOMPOSISI
MIKROBA USUS BESAR DAN KONSENTRASI ASAM LEMAK
RANTAI PENDEK PADA TIKUS SPRAGUE-DAWLEY TERINDUKSI
1,2-DIMETILHIDRAZINE***

MASTER THESIS

by:

WEBIANA LOWISIA (魏碧娜)

17.I3.0006 / 610711015

Submitted in fulfillment of the requirement for Master's degree in Faculty of
Agricultural Technology and College of Science



**DEPARTMENT OF FOOD TECHNOLOGY
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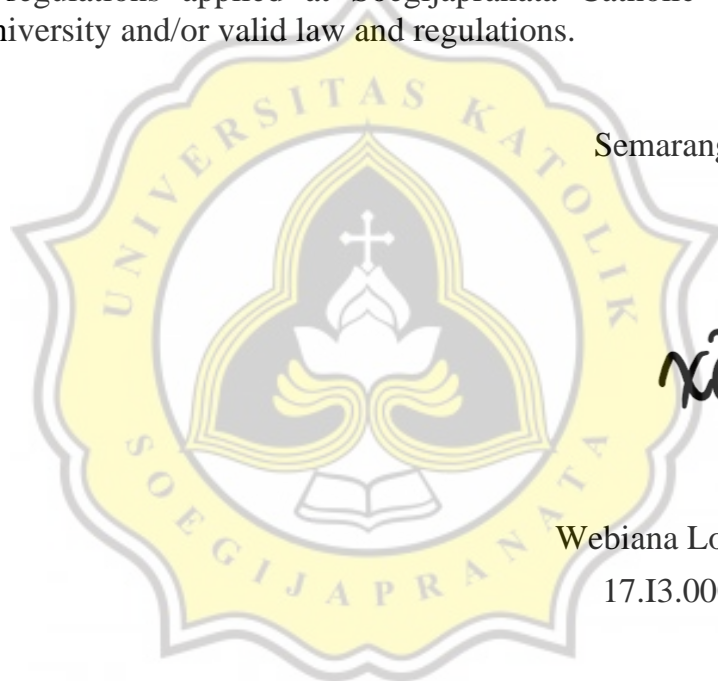
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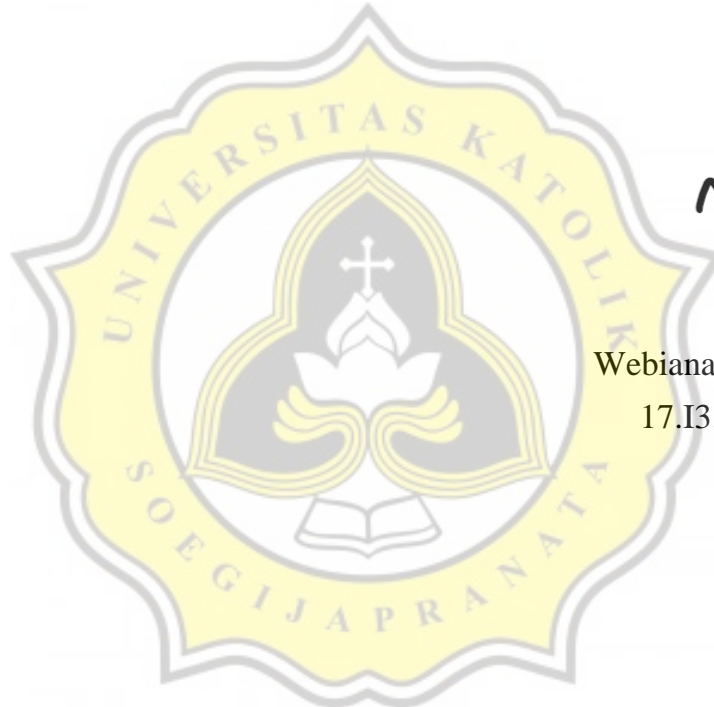
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**This thesis has been approved and defended in front of the examination committees
on June 23th 2020.**

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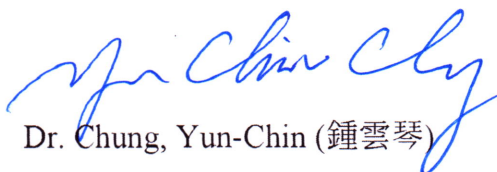
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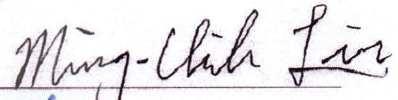
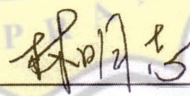
嘉寶果 95%酒精萃取物對 1,2-DIMETHYL-HYDRAZINE 誘導之大
鼠的腸道菌相和短鏈脂肪酸產生的調節作用

**MODULATION OF GUT MICROBIOTA COMPOSITION
AND SHORT CHAIN FATTY ACID PRODUCTION BY
JABOTICABA EXTRACT IN DMH-INDUCED RATS**


Has met the standards of qualifications for a Master's Degree and has thus been approved by this panel.

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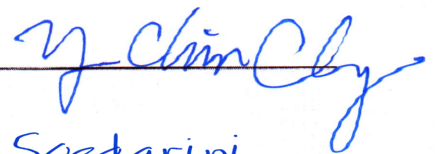
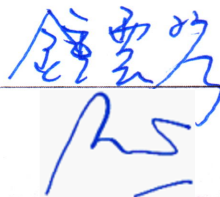
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SUMMARY

Bioactive compounds, such as anthocyanin, play a crucial role in modulating gut microbiota and its metabolites, especially short chain fatty acids (SCFA). Ethanolic extract (95% ethanol) of Jaboticaba (*Myrciaria jaboticaba*) fruits containing high amounts of bioactive compounds were investigated in regard to gut microbiota modulation on a cancer rat model. Six-weeks old Sprague-Dawley male rats were induced colorectal cancer by subcutaneous injection of 1,2-dimethyl-hydrazine (DMH, 30 mg/kg body weight) twice a week for the first two weeks for cancer control and Jaboticaba-treated groups. Rats were gavaged daily with Jaboticaba fruit extract in a high, medium and low dose of 1.0, 0.5, 0.1 g/kg body weight, respectively. Each group was treated for 8 weeks before being sacrificed by cardiac puncture. Gut microflora of the cecum was measured by 16sRNA metagenomics analysis and short chain fatty acid of cecum and fecal content was determined by gas chromatography. There was no significant difference within groups on the pH values ($p < 0.05$), in which pH of cecum and fecal content was in the range of 6.77 ± 0.14 - 7.19 ± 0.25 and 6.21 ± 0.18 - 6.51 ± 0.62 , respectively. Comparing cecum's SCFA concentrations among groups, DMH induction lowered butyrate acid concentration ($p < 0.05$) but neither did acetic acid nor propionic acid ($p < 0.05$). There was no significant difference between normal control and Jaboticaba-treated group, regardless of the applied dosages ($p < 0.05$). For SCFA in feces, the SCFA profiles kept constant through the experimental period and no significant difference was found among groups ($p < 0.05$). A rise in the abundance of *Lachnospiraceae* NK4A136, *Lachnospiraceae* and *Ruminococcus 1* (all have an active role in short chain fatty acid production) were observed in Jaboticaba-treated group, especially in the group received low Jaboticaba concentration. LEFSe (Linear discriminant analysis effect size) revealed that oral admission of Jaboticaba extract at a low dosage showed the highest abundance of *Faecalibacterium*, one of the most abundant and important commensal bacteria of the human gut microbiota which produce butyrate and other short-chain fatty acids through the fermentation of dietary fiber. On the other hand, *Lachnoclostridium*, a biomarker for colorectal cancer, was expressed in high abundance on DMH-induced group and reduced in all Jaboticaba-treated groups. Overall, oral admission of low dose Jaboticaba extract could improve the composition of gut microbiota by stimulating the growth of the mentioned beneficial gut microbiota.

摘要

嘉寶果 (Jaboticaba, 學名 *Myrciaria jaboticaba*) 中富含花青素等具生物活性之植化
物, 本研究旨於探討嘉寶果果實是否能透過調節腸道菌群及其代謝產物達到抑制
腸癌之功能。大鼠模型中腸道微生物群的調節作用。每天用六種不同濃度 (分別
為高, 中, 低劑量, 分別為 1.0、0.5、0.1 g / kg 體重) 的嘉寶果果實之 95%酒精取
物灌胃六週齡之 Sprague-Dawley 雄性大鼠。實驗第一及第二兩週, 每週兩次皮下
注射 1,2-dimethyl-hydrazine (DMH, 30 mg / kg 體重) 誘發大腸癌。每組給於樣品
連續 8 週, 對照組和陽性對照組均用雙蒸餾水給藥, 飼養期結束, 以心臟穿刺犧
牲並採取血液。以 16sRNA 基因定序分析盲腸的腸道菌群。使用氣相層析測量盲
腸內容物及和糞便中 pH 值及短鏈脂肪酸含量 (每兩週收集一次)。盲腸內容物和
糞便 pH 值在每組之間均無顯著性差異, pH 範圍分別為 $6.77\pm 0.14 - 7.19\pm 0.25$ 和
 $6.21\pm 0.18 - 6.51\pm 0.62$ ($p<0.05$)。比較各組盲腸內容物的短鏈脂肪酸濃度, DMH 誘導
會顯著降低丁酸濃度 ($p<0.05$), 但乙酸和丙酸均未降低 ($p<0.05$)。無論使用
何種劑量, 正常對照組和嘉寶果治療組之間均無顯著差異 ($p<0.05$)。對於糞便
中的短鏈脂肪酸, 各個短鏈脂肪酸濃度在整個實驗期間保持恆定, 且各組之間無
顯著差異 ($p<0.05$)。嘉寶果治療組中 *Lachnospiraceae* NK4A136 ,
Lachnospiraceae 和 *Ruminococcus 1* (均為主要產生短鏈脂肪酸之菌株) 的豐富度
提升, 特別是在嘉寶果低劑量組。LEFSe (Linear discriminant analysis effect size)
分析顯示, 嘉寶果低劑量組的 *Faecalibacterium* 含量最高 (*Faecalibacterium* 是人類
腸道菌群中最豐富和最重要的共生細菌之一, 其能發酵膳食纖維產生丁酸和其他
短鏈脂肪酸)。另一方面, *Lachnoclostridium* (大腸癌的生物標誌物) 在 DMH 誘導
組中呈現高豐富度, 而在所有嘉寶果治療組中均降低。總體而言, 餵予大鼠嘉寶
果萃取物能夠藉由刺激上述腸道有益菌群的生長以改善腸道菌群的組成。

RINGKASAN

Antosianin sebagai salah satu senyawa bioaktif utama diduga memiliki potensi dalam mempengaruhi pertumbuhan mikroba tertentu pada usus besar serta mempengaruhi produksi metabolit bakteri, salah satunya yakni asam lemak rantai pendek. Untuk menganalisis hal ini, ekstrak alkohol (95%) dari Jaboticaba yang tinggi dalam senyawa bioaktif, terutama antosianin diaplikasikan terhadap tikus Sprague-Dawley yang telah diinduksi untuk kanker usus besar. Induksi dilakukan dengan injeksi 1,2 dimetilhidrazine (DMH) melalui injeksi subkutan (30 mg/kg berat badan) sebanyak dua kali pada dua minggu pertama pada kelompok kontrol kanker dan kelompok perlakuan Jaboticaba. Ekstrak Jaboticaba dalam 3 konsentrasi yang berbeda, rendah, menengah, tinggi (0.1, 0.5, 1 g/kg berat badan) diberikan melalui oral gavage pada grup Jaboticaba. Perlakuan diberikan selama 8 minggu dan kemudian dikorbankan melalui cardiac puncture. Komposisi mikroba pada cecal kemudian dianalisa dengan menggunakan analisa 16sRNA metagenomik sedangkan konsentrasi asam lemak rantai pendek pada cecal dan fecal dihitung menggunakan gas kromatografi. Hasil menunjukkan tidak ada perbedaan yang signifikan pada pH cecal dan fecal ($p < 0.05$) dengan kisaran $6.77 \pm 0.14 - 7.19 \pm 0.25$ untuk cecal dan $6.21 \pm 0.18 - 6.51 \pm 0.62$ pada fecal. Konsentrasi asam butirat, salah satu asam lemak rantai pendek, lebih rendah secara signifikan ($p > 0.05$) pada bagian cecal dari kelompok kontrol namun perbedaan tidak ditemukan pada asam lemak rantai pendek lainnya dan pada fecal. Peningkatan signifikan pada bakteri Lachnospiraceae NK4A136, Lachnospiraceae dan Ruminococcus 1 (ketiganya merupakan bakteri penghasil asam lemak rantai pendek) dapat diamati pada kelompok Jaboticaba terutama pada kelompok yang menerima Jaboticaba dalam konsentrasi rendah. Uji LEFSe (Linear discriminant analysis effect size) menunjukkan tingginya Faecalibacterium pada kelompok dengan perlakuan konsentrasi rendah. Bakteri ini merupakan salah satu bakteri utama pada usus besar dan berperan dalam menghasilkan asam butirat dan asam lainnya. Selain itu, salah satu bakteri yang digunakan sebagai biomarker untuk kanker usus besar, Lachnoclostridium, ditemukan pada jumlah yang tinggi pada kelompok kontrol kanker dan jumlahnya menurun dengan pemberian Jaboticaba. Melalui hasil yang ada, pemberian Jaboticaba pada konsentrasi rendah menunjukkan potensinya dalam mempengaruhi komposisi mikroba usus besar terutama terhadap bakteri probiotik.

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The author realizes that there were unintended errors in writing this report. The author really allows all readers to give suggestions to improve its content. However, the author hopes that this report can be an inspiration and provide useful information for others.

Semarang, June 1th 2020
Author,



Webiana Lowisia (魏碧娜)

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